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Nina Wurzburger · Anthony S. Hartshorn · Ronald L. Hendrick

Ectomycorrhizal fungal community structure across a bog-forest ecotone in southeastern Alaska

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Abstract We examined the ectomycorrhizal (ECM) fungal community across a bog-forest ecotone in southeastern Alaska. The bog and edge were both characterized by poorly drained Histosols and a continuous layer of *Sphagnum* species, ericaceous shrubs, *Carex* species, and shore pine [*Pinus contorta* Dougl. ex Loud. var. *contorta*]. The forest had better-drained Inceptisols and Spodosols, a tree community comprised of western hemlock [*Tsuga heterophylla* (Raf.) Sarg.], yellow cedar (*Thuja plicata* Donn ex D. Don.), Sitka spruce [*Picea sitchensis* (Bong.) Carr.] and shore pine, and an understorey of ericaceous shrubs and herbs. ECM root tip density (tips cm^{-3} soil) was significantly greater in the forest than the edge or bog and ECM colonization was significantly different in all three plant communities. The below ground ECM fungal taxa were analyzed using molecular techniques (PCR-RFLP and DNA sequencing). Three ECM fungal taxa, *Suillus tomentosus* (Kauffman) Singer, *Cenococcum geophilum* Fr.:Fr, and a *Russula* species, differed in relative frequency, yet were among the four most frequent in all three plant communities. Although differences in ECM fungal richness were observed across plant communities, unequal sampling of ECM roots due to root density and colonization differences confounded richness comparisons. Using resampling procedures for creating taxon-accumulation curves as a function of sampled ECM roots revealed similarities in cumulative ECM fungal taxa richness across the ecotone.

N. Wurzburger (✉) · R. L. Hendrick
Warnell School of Forest Resources,
University of Georgia,
Athens, GA 30602–2152, USA
e-mail: wurzburg@uga.edu
Tel.: +1-706-5421385
Fax: +1-706-5428356

A. S. Hartshorn
Department of Geography,
University of California,
Santa Barbara, CA 93106–4060, USA

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Introduction

The islands of the southeast Alaskan panhandle are characterized by a heterogeneous landscape, including peatlands on poorly drained sites and forests on better-drained sites. Peatlands in this region have expanded and contracted in response to wetter and drier climatic periods, respectively (Heusser 1952; Klinger et al. 1990; Hansen and Engstrom 1996). The boundaries between peatlands and forests have been suggested to be under constant tension because they are sensitive to changes in local hydrology (Neiland 1971). One consequence, reported by Hartshorn et al. (2003), is an apparent soil-vegetation uncoupling at a peatland-forest boundary. For example, they found that soil characteristics at the forest edge were remarkably similar to that of the bog, yet overstorey stem basal areas were similar to that of the forest. In addition, large differences in soil respiration along the ecotone did not coincide with the large differences in tree basal areas.

Soil and hydrological characteristics typifying these peatland-forest ecotones may determine how the individual plant communities respond to changes in precipitation and temperature, but there are few data upon which to base such predictions. Complex interactions are likely to occur between soil taxonomic features, water table depth, nutrient availability, soil and root respiration, and root distributions across peatland-forest boundaries. Mycorrhizal fungi may also be affected by these factors, especially the ectomycorrhizal (ECM) fungi that are characteristic of boreal forests. ECM host trees in the genera *Pinus* and *Picea* often dominate boreal forests, and ECM fungi are similarly dominant; ECM root tip abundance ranges from 10^4 to 5×10^5 m^{-2} (Dahlberg et al. 1997; Dahlberg 2001), and ECM fungal tissue can comprise one-third of soil microbial biomass (Högberg and Högberg 2002).

ECM fungi are integral to forest function, most notably for their important role in nutrient acquisition in soils of low fertility (Smith and Read 1997). ECM fungi possess a diverse array of nutrient-acquiring characteristics, from the production of enzymes that catabolize proteins and organic P (Colpaert and Van Laere 1996; Antibus et al. 1997) to the capacity for uptake of amino acids (Chalot and Brun 1998). ECM fungi are intimately linked to both their plant host and the soil environment; host specificity of ECM fungi as well as soil characteristics can influence the structure of ECM fungal communities (Molina et al. 1992; Gehring et al. 1998). For example, changes in ECM fungal community richness and composition across an alpine/subalpine ecotone were related both to host plant composition and edaphic characteristics (Kernaghan and Harper 2001).

The response of ECM fungal communities to changes in soil hydrology associated with peatland-forest gradients is not known. In fact, very little is known about ECM fungal communities in forested wetlands in general. ECM fungi are sensitive to soil aeration and oxygen availability, and there is evidence that ECM colonization decreases with increased soil moisture (Lodge 1989). However, other surveys have revealed that ECM trees are indeed colonized by ECM fungi in wetland habitats (Glenn et al. 1991; Thormann et al. 1999), including situations of permanent inundation (Baar et al. 2002).

We examined ECM roots and the ECM fungal community across a well-characterized peatland-forest ecotone (Hartshorn et al. 2003). The objectives of this study were to compare characteristics of ECM fungi (ECM root tip density and ECM colonization), as well as to identify the members of the ECM fungal community and compare community composition (β diversity) and richness, in three plant communities across a peatland-forest ecotone.

Materials and methods

Site description

We characterized the ECM fungal community across a peatland-forest ecotone (Common Snipe, 56°34′N, 132°50′W, elevation 17 m, Hartshorn et al. 2003) on Mitkof Island (500 km^2), which has one of the largest fractions of peatlands (hereafter, bogs) of any large island in southeastern Alaska (Dachnowski-Stokes 1941; USDA 1997). Air temperatures average −1.2°C in winter and 12.6°C in summer, with an annual average of 5.7°C. Annual rainfall averages 3,700 mm, with a mean annual snowfall of 2,600 mm (Hogan 1995). On Mitkof Island, soils across bog-forest gradients range from Typic Cryohemists in poorly drained settings to Oxyaquic Haplocryods in better-drained settings (Hartshorn et al. 2003).

The bog and edge were both characterized by an understorey of ericaceous shrubs, *Carex* spp., and a continuous layer of *Sphagnum* spp. *Pinus contorta* Dougl. ex Loud. var. *contorta* (shore pine) grew stunted (<3 m) in the bog and taller along the edge (~8 m) and was interspersed with *Chamaecyparis nootkatensis* (ex Don) Spach. (Alaska yellow cedar). Adjacent forests are of similar height as the edge and are dominated by *Tsuga heterophylla* (Raf.) Sarg. (western hemlock), *Picea sitchensis* (Bong.) Carr. (Sitka spruce), *P. contorta*, *C. nootkatensis* and *Thuja plicata* Donn ex D. Don. (western red cedar). Because of the improved drainage associated with incised creeks and rivers, riparian forests occupy some of the lowest elevations on the landscape, and at our site the forest was 1.2 m lower than the adjacent bog.

Root sampling

We sampled from bog, edge and forest communities along three ecotone transects in an area of approximately 1 ha. We randomly established three transect locations, each comprised of the same dominant vegetation described above, along a 100 m length parallel to the forest edge. Transects were oriented perpendicular to the forest edge boundary. Along each transect (approximately 100 m in length), one sampling plot was established within each zone of the ecotone (bog, edge and forest). Plot centers were chosen randomly in the edge and forest (because of the relatively even distribution of trees and complete canopy closure), while plots were centered on areas colonized by *P. contorta* in the bog, where tree distribution was patchy. In addition, we avoided sampling in standing water in the bog, as these low areas lacked ECM roots. In each plot we randomly chose three cores from a grid of 17 points (nine locations at a 1.5×1.5 m spacing, and an additional eight locations nested within these at a 0.25×0.25 m spacing). Across transect 1, an additional 14 cores (i.e., all 17 cores) were sampled from each of the three plots. We attempted to sample equal numbers of fine roots from the bog, edge and forest. Because root density varied along the transect, core dimensions were therefore 5×5×10 cm in depth in the bog, 5×5×5 cm in depth in the edge, and 2.5×2.5×5 cm in depth in the forest.

Core processing

From each core we separated ECM roots (of *P. contorta*, *T. heterophylla* and *P. sitchensis*) from *Sphagnum*, organic matter, and other plant roots. These roots are easily distinguished from the hair roots and fibrous roots of sedges and ericaceous plants. Non-mycorrhizal and dead roots were quantified by numbers of root tips, while ECM roots were further separated by morphotypes based on mantle color and texture, the presence and characters of extramatrical hyphae, and macro- and micro-characters of the ECM mantle (Agerer 1991). ECM frequency (number of tips or individual tubercle clusters) was determined for each morphotype per soil core, and a subsample of tips was frozen within 7 days of core sampling. Frequency values for each morphotype were also classified into two host tree groups (*P. contorta* or *T. heterophylla* and *P. sitchensis*) based on ECM root branching patterns. Since *Pinus* ECM roots are typically dichotomous and coralloid (Agerer 1991), we used these characters (in contrast to pinnate branching) to distinguish *Pinus* roots from the other ECM hosts. Where ECM roots were unbranched or not uniformly dichotomous, we classified the ECM roots based on branching patterns of other ECM morphotypes attached to the same root. In addition, ECM colonization (% root tips colonized by ECM fungi) and ECM root tip density (ECM root tips cm^{-3} soil) were quantified from each core.

Molecular identification of ECM fungi

We used a combination of morphotyping and molecular identification to characterize ECM root tips. Our molecular identification methods involved typing the internal transcribed spacer (ITS) region of nuclear DNA by restriction fragment length polymorphisms (RFLP) (Gardes and Bruns 1996), followed by ITS sequencing and taxa identification (see below for details). At least one root tip of each morphotype group from each core was used for DNA extraction and molecular identification. Out of the 8,619 ECM root tips that were sampled and morphotyped, 236 were used for molecular identification. In order to test for taxonomic integrity of within-core morphotype groups we processed one or two additional tips from 25 randomly selected morphotypes. Of these, 97% of the root tip ITS types were identical to the other root tips in that group (N. Wurzburger, unpublished data), evidence that one

root tip per morphotype group per core was sufficient for identification.

DNA was extracted from lyophilized root tips (Gardes and Bruns 1996) and the ITS region was amplified by the primers ITS1-F and ITS4 (White et al. 1990). RFLP digests of the restriction enzymes *Alu*I and *Hinf*I were used to distinguish ITS types (band sizes were quantified with Gel Imager, Alpha Innotech, San Leandro, Calif.). Representative samples from each ITS type were reamplified, purified (Qiaquick PCR purification kit, Qiagen, Valencia, Calif.) and sequenced (ABI Big Dye terminator kit, analyzed on a ABI model 310 DNA sequencer, Perkin-Elmer, Foster City, Calif.). Sequences were analyzed with SeqMan II software (DNAstar, Madison, Wis.) and compared to GenBank sequences for taxonomic identification [BLAST (basic local alignment software tool)]. Those with $\geq$99% similarity were considered a match for species identification while the remaining samples were classified by appropriate taxonomic groups.

Analyses

ECM root tip density (ECM root tips cm^{-3} soil) and ECM colonization were tested for normality, transformed, and analyzed with a one-way ANOVA across bog, edge and forest plant communities and blocked by transect. The relative frequency of each ECM fungal taxon (expressed as a percentage) was calculated at each plant community by dividing the number of root tips of that taxon by the total number of ECM root tips sampled in the community. We compared the β diversity of ECM fungal taxa composition in each plant community with two similarity indices, the Sørensen (Cs) and Sørensen quantitative (Cn) indices:

$$Cs = 2j/(a+b) \quad (a)$$

where j=the number of species found in both sites, a=the number of species in site A, b=the number of species in site B;

$$Cn = (2jN)/(aN+bN) \quad (b)$$

where jN=the sum of the lower of the two frequencies recorded for species found in both sites, aN=the total number of individuals (root tips) in site A and bN=total number of individuals in site B. For both indices, values closer to 1 indicate more similarity between sites (Magurran 1988). To further visualize ECM fungal richness across the ecotone, we constructed ECM fungal taxa-accumulation curves by resampling (1,000 times, with replacement) species richness from our randomized sample data, using the computer program EstiMateS (Colwell 1997). We used 95% confidence intervals of each of the resampled means to make comparisons of taxon accumulation between the three plant communities along the ecotone.

Results

ECM colonization and density

We sampled a total of 9,994 root tips (mycorrhizal and non-mycorrhizal) of *P. contorta*, *T. heterophylla* and *P. sitchensis* from three transects across the gradient. Despite our attempt to account for differences in rooting densities by altering sample core volumes 8-fold, we sampled only one-fifth of the number of ECM root tips in the bog compared to the edge and forest (Table 1). ECM root tip density (ECM tips cm^{-3} soil) was significantly greater (P<0.05) in the forest compared to the bog and edge with a block effect (P=0.02). ECM colonization (no block effect) increased from bog to forest, and was significantly different in all three plant communities (Table 1).

ECM fungal community structure and richness

We identified 10, 22 and 21 morphotypes corresponding to 9, 14 and 17 DNA-derived ECM fungal taxa in the bog, edge and forest, respectively. From these morphotypes, we observed 26 unique ITS types, while 3 morphotypes remain uncharacterized. Bog, edge and forest morphotypes produced 7 ITS types (and two morphotypes), 13 ITS types (and 1 morphotype) and 17 ITS types, respectively. Of the 26 ITS types, 20 were classified to a genus or family, five of these identified to species based on comparisons to ITS sequences in GenBank. Twenty-two of the ECM fungal taxa were Basidiomycetes and of the four Ascomycetes, only *Cenococcum geophilum* Fr.:Fr had a relative frequency greater than 10% in any plant community. The Cortinariaceae was the most represented ECM family with eight taxa: one *Cortinarius* sp., two *Hebeloma* spp., three *Dermocybe* spp., and two taxa of undetermined genus (Cortinarioid 1 and 2). Four ECM fungal taxa (*Suillus tomentosus* (Kauffman) Singer, *C. geophilum*, *Russula* sp. and *Clavulina* sp. 1) were observed in all three plant communities, and three of these taxa were among the four most abundant taxa in each plant community. Besides these four shared ECM fungal taxa, the edge and forest both contained Helotiales 1 and *Tomentellopsis* sp., and the bog and edge had one other taxon in common (*Dermocybe* sp. 1) (Fig. 1). We classify the taxa we observed in the Helotiales (Helotiales 1 and 2) as putative ECM taxa because the morphotype associated with these taxa possessed a fungal mantle; however, we did not observe a Hartig net.

All three plant communities were characterized by one dominant (>45%) and several infrequent ECM fungal taxa (Fig. 1). Host tree ECM root abundance reflected the distribution of tree species across the ecotone. ECM roots

Table 1 Comparison of roots sampled, ectomycorrhizal (ECM) colonization and ECM root tip density in locations along the ecotone[a]

	Bog	Edge	Forest
ECM root tips cm^{-3} soil	0.05 (0.03) a	1.92 (0.2) a	6.04 (1.2) b
Total root tips sampled[b]	1,553	4,422	4,019
ECM root tips sampled	779	3,852	3,988
ECM (%)	53 (8) a	88(3) b	99(0.4) c

[a] All data are from 24 cores sampled in each location. Mean values and (SE) followed by a different letter in the same row are significantly different (P<0.05)

[b] Live mycorrhizal and nonmycorrhizal root tips of *Pinus contorta*, *Tsuga heterophylla* and *Picea sitchensis*

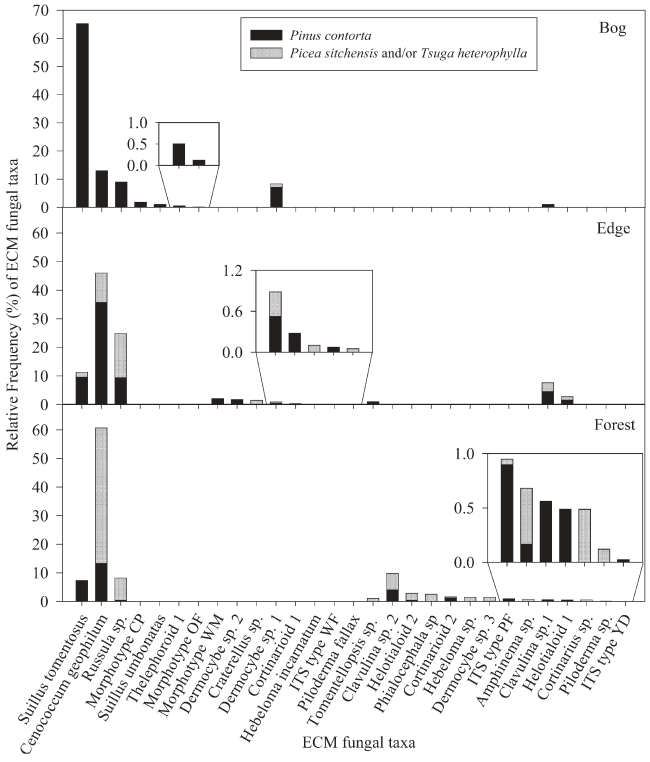

Fig. 1 Relative frequency of ectomycorrhizal (ECM) fungal taxa by host tree group in bog, edge and forest locations. Relative frequency =[(number of ECM roots)/(total number of ECM roots per location)]×100. Bars for fungal taxa with a relative frequency of less than 0.8% are enlarged for clarity

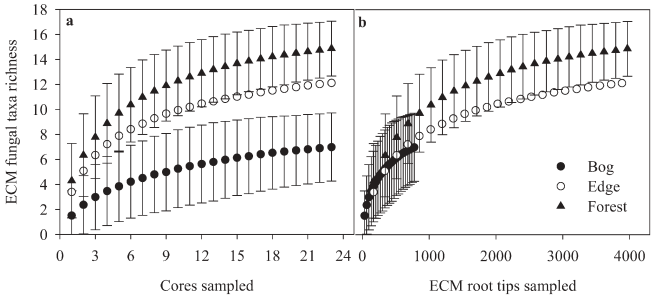

Fig. 2a, b ECM fungal taxon-accumulation curves from the bog, edge and forest constructed by resampling richness (1,000 times, with replacement) and expressed by **a** number of cores sampled and **b** number of sampled ECM root tips. Bog and forest 95% confidence intervals of resampled means shown (edge confidence intervals omitted for clarity)

of *P. contorta* dominated samples from the bog and edge while forest co-dominants *T. heterophylla* and *P. sitchensis* accounted for a majority of ECM roots in the forest. All of the ECM fungal taxa that were observed in more than one plant community were also observed on roots from both host tree groups. ECM fungal taxa observed only on one of the host tree groups were observed at relatively low frequency (<5%) and together accounted for <12% of all observed roots (Fig. 1).

The Sørensen index, based on the presence and absence of species at each site, revealed greater similarity between neighboring communities (bog vs edge 0.45, and edge vs forest 0.40) than between bog and forest (0.31). Based upon the results of the Sørensen quantitative index, which includes species frequency, edge and forest (0.64) were more similar than were bog and edge (0.28), or bog and forest (0.20).

We constructed ECM fungal taxon-accumulation curves in order to examine ECM fungal community richness at each plant community. The bog appears to have lower ECM taxa richness (from 95% confidence intervals of the resampled mean) than the forest between core samples 6 through 23 (Fig. 2a), while edge richness was not different from that of the bog or the forest. However, because the number of ECM root tips per core differed in the bog, edge and forest (Table 1), we also expressed the number of ECM taxa as a function of the number of ECM roots sampled (Fig. 2b). There is no apparent difference in richness across the ecotone up to the minimum number of roots sampled in any plant community (779), and none between the edge and forest up to 3,852 sampled roots.

Discussion

ECM fungi in bogs and wetlands

ECM fungi have seldom been studied in bogs and other wetland environments. While bogs are unique in some respects in comparison to many of the upland communities in which ECM fungi occur (e.g., organic soils, high water table, limited aerobic zone), the available evidence indicates that ECM fungi are typically present in these environments. For example, ECM fungi have been observed on bog *P. contorta* (this study), *Picea rubens* Sarg. (including *C. geophilum*, Glenn et al. 1991) and *Betula pubescens* Ehrh. (Tomassen et al. 2003). In Canadian peatlands, Thormann et al. (1999) observed *C. geophilum* on roots of *Picea mariana* (Mill.) B.S.P., as well as sporocarps of *Cortinarius*, *Lactarius*, and *Russula* species. However, little is known about the diversity of ECM fungi in wetland environments relative to drier, upland forest communities. In spruce forests of the Karelian Isthmus, Russia, lower ECM fungal sporocarp richness was observed in bogs compared to upland habitats (Fomina 2001). Individual rare ECM morphotypes on *P. rubens* were observed less frequently in bogs relative to upland habitats (Glenn et al. 1991). While in our study ECM fungal richness in the bog was nearly half that of the forest, normalization of observed ECM fungal taxa relative to sampled root tips suggests no significant difference in richness across the ecotone (see below).

The composition of ECM host trees can influence ECM fungal community composition and diversity (Kernaghan et al. 2003), possibly due to the host specificity of some ECM fungi (Molina et al. 1992). This phenomenon may be an important consideration with ECM fungal

communities in wetlands, as some ECM hosts are successful across a variety of hydrologic regimes, while others have a more limited distribution. For example the genera *Picea*, *Pinus* (e.g., *P. contorta* in this study), and *Betula* contain species that associate with a large number of ECM fungi (Molina et al. 1992) and readily colonize both peatlands and uplands (Harlow et al. 1996). In contrast, *Alnus glutinosa* (L.) Gaertn. specializes in lowland habitats in Europe and, although it is heavily colonized by ECM fungi, appears to associate with a limited number of ECM fungal taxa (Baar et al. 2000, Baar 2002).

The abiotic or edaphic characteristics of soils also influence the composition and diversity of ECM fungal communities. Oxygen availability, in particular, is likely to affect the growth and distribution of ECM roots and fungi in wetland habitats. Trees in peatlands tend to have shallow rooting depths (Håland and Brække 1989; Brække 1992) coinciding with the zone of aerobic activity (Urban and Eisenreich 1988). Hummock or mound microtopography in bogs, wetlands and hydric forests have been associated with concentrations of fine root biomass and higher levels of mycorrhizal colonization, attributed to greater oxygen availability (Glenn et al. 1991; Cantelmo and Ehrenfeld 1999; Wurzburger and Bledsoe 2001). In this study, ECM root depth appears to be influenced by the depth of the water table. All ECM roots in the bog and edge were observed within 5 cm of the bog surface (typically the living layer of *Sphagnum*), corresponding to the depth of the water table (~4 cm, 1999–2001 average depth, Hartshorn et al. 2003). In contrast, the forest had comparatively better drainage (water table depth ~17 cm, Hartshorn et al. 2003) and ECM roots were observed to a depth of at least 10 cm. However, ECM roots and fungi are not necessarily limited to aerobic soil layers. For example, *A. glutinosa* is colonized by ECM fungi despite growing in areas of permanent inundation (Baar et al. 2000). The ECM fungal hyphae growing in anaerobic soils may be acquiring oxygen diffused from its associated roots and/or roots of neighboring plants (Read and Armstrong 1972; Baar et al. 2002). Clearly, there is a need to better understand the mechanisms by which ECM fungi cope with limited oxygen availability in wetland habitats, not only to explain the distribution and colonization of ECM roots and ECM fungi, but also to elucidate their functioning.

ECM colonization and root tip density

ECM colonization of *P. rubens* was not different in lowland bogs compared to mesic upland soils (Glenn et al. 1991). However, in our study, ECM colonization and ECM root tip density were substantially lower in the bog than in the adjacent forest. In addition, despite the similarity in soils, hydrology and ECM tree host (*P. contorta*), ECM colonization was lower in the bog than the edge. Greater than 90% of the fine root tips of most coniferous ECM hosts are colonized by ECM fungi (Smith and Read 1997; Taylor et al. 2000); however, low levels (~20–50%) of ECM colonization were also reported of *Pinus edulis* Engelm. in northern Arizona (Gehring and Whitham 1994). Lower levels of ECM colonization at one *P. edulis* site appeared to be associated with a trend of greater herbivory (Gehring and Whitham 1994), highlighting the possibility that the carbon budget of the plant host may influence ECM colonization, a carbon cost to the host plant (Newman 1988). In our study, tree productivity (estimated from basal area and tree age, Hartshorn et al. 2003) is dramatically lower in the bog compared to the forest and could be driving the observed differences in ECM colonization.

ECM fungal community structure

Differences in ECM fungal community composition across the ecotone we studied are likely to be due, in part, to host tree composition. Although over half of the ECM fungal taxa were only observed on one host tree group, the frequency of these taxa was low (<5%). Consequently, it is not clear if these ECM fungal taxa are indeed host specific or, rather, simply not observed on roots of other hosts because they are infrequent (an issue raised by Horton and Bruns 1998 and Cullings et al. 2000).

In spite of changes in soils and ECM host composition, the same three ECM fungal taxa were among the four most dominant across the ecotone. Similarly, Glenn et al. (1991) observed the same three dominant ECM morphotypes on *P. rubens* in both bogs and upland soils. *C. geophilum* colonizes a wide array of hosts in a number of different habitats (Molina et al. 1992; LoBuglio 1999). It was the dominant ECM fungal taxon in the edge and forest (47 and 62%, respectively), and was the most dominant ECM taxon in 16 out of 27 reports from Canada and Sweden (summarized in Dahlberg 2001).

In our study, *S. tomentosus* displayed preference to *P. contorta*, although it was also observed at low frequencies on roots of *T. heterophylla* and/or *P. sitchensis*. The host preference of *S. tomentosus* and the distribution of *P. contorta* across the ecotone we studied may be responsible for the decrease in its frequency from bog to forest. *Suillus* is purportedly host specific to Pinaceae, and *S. tomentosus* to the genus *Pinus* (Molina et al. 1992; Dahlberg and Finlay 1999). However, there have been reports of *S. tomentosus* on *Picea engelmannii* Parry ex Engelmann. (Cullings et al. 2000), and a *Suillus*-like morphotype was observed on outplanted seedlings of *Picea glauca* (Moench) Voss and *Abies lasiocarpa* (Hook.) Nutt. (Kranabetter et al. 1999). In addition, the vegetative nature of *Suillus* could also be responsible for its dominance in our study. *Suillus* tends to be prevalent in older forest stands (Visser 1995) perhaps in part because it forms large, continuous genets, some with an area equal to one-third the sampling area of our study (Dahlberg 1997; Bonello et al. 1998).

ECM fungal richness

ECM fungal richness increased from the bog to the forest, and ECM fungal community similarity was greater between adjacent plant communities along the ecotone (bog and edge, or edge and forest). However, comparisons of the ECM fungal community structure and/or richness between plant communities are contingent upon equal sampling of the communities. Although we sampled equal numbers of cores, differences in ECM colonization and ECM root density across the ecotone resulted in unequal sampling of ECM roots, making richness comparisons problematic (see also Taylor 2002). The log-normal abundance distribution of ECM fungal communities (Horton and Bruns 2001) contributes to the difficulty of sampling them. Across the ecotone, the most abundant ECM fungal taxon represented at least 47% of ECM root tips, and the three most abundant taxa, in combination, accounted for at least 90% of all sampled root tips. The more abundant the dominant taxa become, the more difficult it is to detect less abundant taxa (Taylor 2002), and in our study ECM fungal taxa that were infrequent (<1% of roots) accounted for most of the differences in ECM fungal community composition.

Since ECM fungal communities are virtually impossible to sample in their entirety, species-accumulation curves have been used to examine how sampling intensity influences observations of ECM fungal richness (Horton and Bruns 2001). Recently, Taylor (2002) constructed ECM fungal taxon-accumulation curves to illustrate how differences in root tip density between sites can misguide comparisons of ECM fungal richness. We constructed ECM fungal taxa-accumulation curves expressed both by number of samples (Fig. 2a) and number of root tips examined (Fig. 2b). From the number of ECM roots sampled in the bog, we have no evidence to suggest a difference in ECM fungal taxa richness across the ecotone. It is possible that the forest has greater ECM fungal richness than the bog; however, detecting this difference may require sampling a greater number of ECM roots.

In summary, ECM colonization paralleled changes in basal area across the ecotone (Hartshorn et al. 2003), with the sharpest contrast between bog and edge communities, despite the absence of significant differences in soil and hydrologic features. Because differences in tree basal area, tree height and tree age were most dramatic between the bog and the edge (Hartshorn et al. 2003), these findings suggest that lower productivity associated with the stunted trees colonizing the bog may result in reduced ECM colonization of root tips. We found the same three (*S. tomentosus*, *C. geophilum* and a *Russula* sp.) relatively abundant ECM taxa at bog, edge and forest locations even though the dominant ECM taxa shifted from *S. tomentosus* in the bog to *C. geophilum* in the edge and forest. We observed an increase in ECM fungal taxa richness from bog to forest; however, unequal sampling of ECM roots confounded comparisons. Taxon-accumulation curves expressed by the number of sampled ECM roots revealed no differences in richness across the ecotone, although our comparisons were limited by the minimal number of ECM roots sampled in the bog. These results highlight the difficulty in comparing ECM fungal richness between studies, sites or different soil horizons based upon numbers of soil cores when root densities and/or ECM colonization are not similar. More appropriate sampling strategies of ECM roots (e.g., stratified single root samples, Taylor 2002) or extracting ECM fungal DNA from the soil for analysis (e.g., T-RFLP analysis, Dickie et al. 2002) in order to identify ECM fungal mycelia in conjunction with root tips, would prevent the bias involved in relying solely on root colonized ECM fungi from soil cores in order to sample the ECM fungal community.

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